Article

Bio-Inspired Nitrile Hydration by Peptidic Ligands Based on L-Cysteine, L-Methionine or L-Penicillamine and Pyridine-2,6-dicarboxylic Acid

Cillian Byrne [†], Kate M. Houlihan [†], Prarthana Devi, Paul Jensen and Peter J. Rutledge *

School of Chemistry F11, The University of Sydney, Sydney, NSW 2006, Australia;
E-Mails: cilliandbyrne@googlemail.com (C.B.); tynan.kate@gmail.com (K.M.H.);
prarthana.devi@sydney.edu.au (P.D.); paul.jensen.email@gmail.com (P.J.)

[†] These authors contributed equally to this work.

* Author to whom correspondence should be addressed; E-Mail: peter.rutledge@sydney.edu.au;
   Tel.: +61-293-515-020; Fax: +61-293-513-329.

External Editor: Katrina Jolliffe

Abstract: Nitrile hydratase (NHase, EC 4.2.1.84) is a metalloenzyme which catalyses the conversion of nitriles to amides. The high efficiency and broad substrate range of NHase have led to the successful application of this enzyme as a biocatalyst in the industrial syntheses of acrylamide and nicotinamide and in the bioremediation of nitrile waste. Crystal structures of both cobalt(III)- and iron(III)-dependent NHases reveal an unusual metal binding motif made up from six sequential amino acids and comprising two amide nitrogens from the peptide backbone and three cysteine-derived sulfur ligands, each at a different oxidation state (thiolate, sulfenate and sulfinate). Based on the active site geometry revealed by these crystal structures, we have designed a series of small-molecule ligands which integrate essential features of the NHase metal binding motif into a readily accessible peptide environment. We report the synthesis of ligands based on a pyridine-2,6-dicarboxylic acid scaffold and L-cysteine, L-*S*-methylcysteine, L-methionine or L-penicillamine. These ligands have been combined with cobalt(III) and iron(III) and tested as catalysts for biomimetic nitrile hydration. The highest levels of activity are observed with the L-penicillamine ligand which, in combination with cobalt(III), converts acetonitrile to acetamide at 1.25 turnovers and benzonitrile to benzamide at 1.20 turnovers.
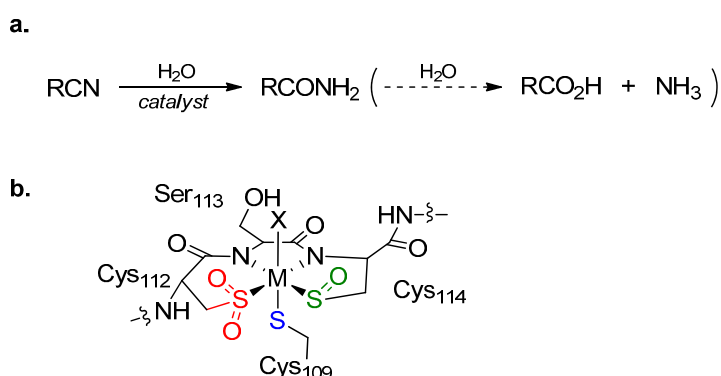
1. Introduction

The development of mild, environmentally benign methods to convert organic nitriles to primary amides (Figure 1a) is of considerable interest given potential applications of these methods in synthesis, bioremediation and the chemical industry [1–3]. Catalytic hydration of nitriles offers an efficient route to amides, which have a wide range of potential applications in polymer manufacture and pharmaceutical synthesis [1,2].

Classical methods for hydrating nitriles require treatment with strong acid or base under forcing conditions which often result in over-hydrolysis to the carboxylic acid [3]. A number of promising systems have been developed in recent years using transition metal catalysis to effect nitrile hydration under increasingly mild and selective conditions [1,2]. Of particular note are recent approaches employing ruthenium [4–7], rhodium [8,9], palladium [10,11], platinum [12,13], copper [14], silver [15] and gold catalysts [16,17]. Transition metal-free strategies have also been reported recently using potassium carbonate [18] or 1,3-dimethylimidazolium hydrogen carbonate as an organocatalyst [19], and anhydrous conversion of nitriles to amides has been achieved using aldoximes as the water source [11,20,21].

Figure 1. (**a**) Nitrile hydration to the primary amide (and onward to carboxylic acid and ammonia); (**b**) The metal binding environment at the active site of Co-NHase [22]. The metal is coordinated by two nitrogen atoms from backbone amides and three sulfur atoms; all the *S*-ligands derive from the side-chain thiols of cysteine residues, but each is in a different oxidation state: thiolate (blue), sulfenate (green) and sulfinate (red). X = solvent.


Biocatalysis offers another efficient route to amides from nitriles [23,24]. The hydro-lyase enzyme nitrile hydratase (NHase, EC 4.2.1.84) is used in the industrial production of nicotinamide and acrylamide [24,25], and is increasingly utilised as a biocatalyst for synthesis [26]. NHase has been well studied, and a variety of mechanistic investigations and high-resolution crystal structures reported [22,23,27,28]. NHase is a metalloenzyme and incorporates iron(III) or cobalt(III) in an unusual coordination sphere (Figure 1b). It was long thought that the active site metal ion either activated water to attack the nitrile or activated the nitrile to attack by water [29,30]. However recent structural studies

by Holz and co-workers [31] and theoretical calculations by Hopman and Himo [32,33] suggest that the cysteine-sulfenic acid ligand is the catalytic nucleophile.

Several groups have used NHase as the inspiration for biomimetic nitrile hydration [29,30,34–40]. A handful of synthetic small-molecule complexes incorporating nitrogen and sulfur ligands have been shown to bind nitriles [41] and to promote nitrile hydration in solution [4,35,37,39]. Building on these previous biomimetic approaches to nitrile hydration and our work with related non-heme iron systems [42–47], we report now the synthesis and evaluation of ligands **1**–**4** based on the pyridine-2,6-dicarboxylic acid scaffold **5** (Figure 2) and the amino acids L-cysteine (**6**), L-*S*-methylcysteine (**7**), L-methionine (**8**) and L-penicillamine (**9**). These compounds introduce a peptide character around the pyridine-2,6-dicarboxamide core to furnish pentadentate N_3S_2 ligands to mimic the NHase active site.

Figure 2. Ligands **1**–**4** prepared and studied in the current investigation are based on pyridine-2,6-dicarboxylic acid **5** and sulfur-containing amino acids.
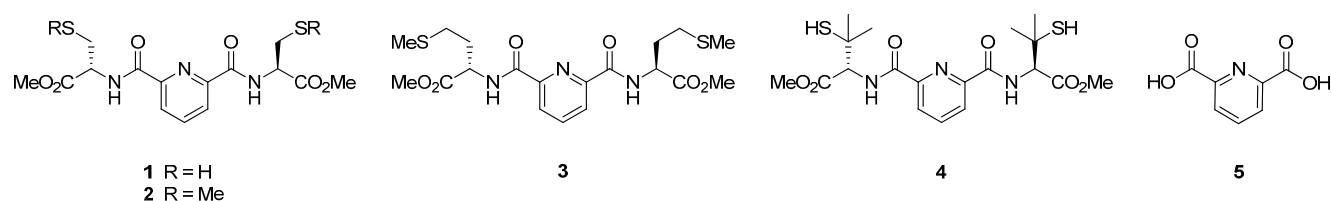

2. Results and Discussion

2.1. Synthesis of Ligands

Ligands **1**–**4** were prepared from pyridine-2,6-dicarboxylic acid (**5**) and L-cysteine (**6**), L-methionine (**8**) and L-penicillamine (**9**) (Scheme 1). The amino acid constituents were derivatised using established procedures for the preparation of *S*-methyl-L-cysteine [48] and *S*-*p*-methoxybenzyl-L-cysteine [49] from L-cysteine (**6**), protection of L-penicillamine (**9**) via the *S*-*p*-methoxybenzyl (PMB) derivative **10** [49], and methyl esterification [50,51]. The methyl esters **11**–**14** were then coupled to the diacid **5** using *N,N,N',N'*-tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU) under standard conditions [52]. This afforded ligands **2** and **3** directly and the *S*-protected forms of ligands **1** and **4**, the *S*-*p*-methoxybenzyl-L-cysteine derivative **15** and *S*-*p*-methoxybenzyl-L-penicillamine analogue **16**. Trifluoroacetic acid-mediated deprotection using triisopropylsilane (TIS) as a cation scavenger was used to convert **15** to ligand **1** and **16** to ligand **4** [49]. The methionine derivative **3** has previously been reported by Deardau *et al.*, who investigated the use of this compound and the pyridine *N*-oxide derivative as a ligand for enantioselective reduction of ketones [53], while compound **1** has been described briefly (without full characterisation details) in a report detailing its use in the preparation of hybrid membranes that incorporate amide receptors [54].

Scheme 1. Synthesis of ligands **1–4** from pyridine-2,6-dicarboxylic acid (**5**) and the amino acids L-cysteine (**6**), L-*S*-methylcysteine (**7**), L-methionine (**8**) and L-penicillamine (**9**).
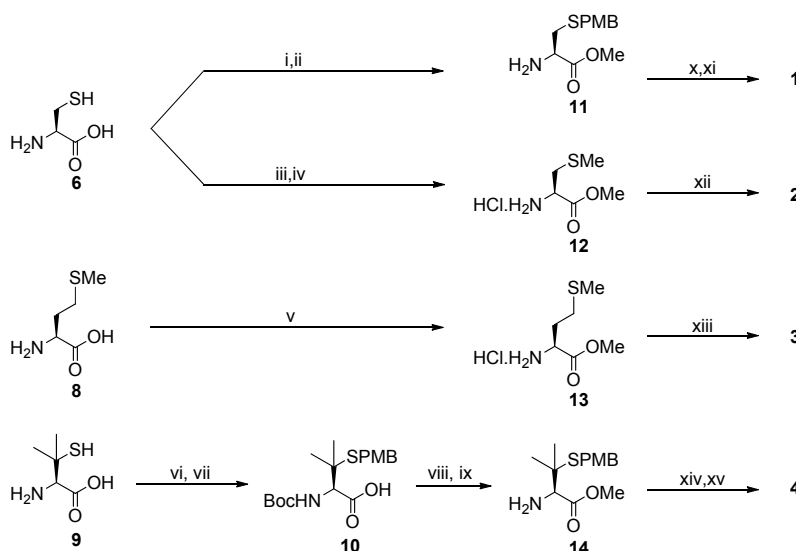
Reagents and Conditions: i. PMBCl, TFA, DCM, 0 °C 1 h then rt 18 h, 93%; ii. SOCl$_2$, MeOH, reflux, 20 h, 77%; iii. Na, MeI, EtOH, 0 °C, 30 min, 85%; iv. SOCl$_2$, MeOH, reflux 4 h then rt 16 h, 100%; v. SOCl$_2$, MeOH, rt 4 h then reflux 20 h, 89%; vi. PMBCl, AcOH, 80 °C, 72 h; vii. Boc$_2$O, Et$_2$^{i}PrN, MeOH, reflux 3 h then rt 16 h, 89% over two steps; viii. NaHCO$_3$, MeI, DMF, rt, 16 h, 62%; ix. *p*-toluenesulfonic acid, CH$_2$Cl$_2$, reflux, 3 h then NaHCO$_3$, 90%; x. pyridine-2,6-dicarboxylic acid **5**, HBTU, Et$_3$N, CH$_2$Cl$_2$, rt, 20 h, 42%; xi. TFA, TIS, reflux, 2.5 h, 63%; xii. **5**, HBTU, Et$_3$N, CH$_2$Cl$_2$, rt, 23 h, 68%; xiii. **5**, HBTU, Et$_3$N, CH$_2$Cl$_2$, 24 h, rt, 33%; xiv. **5**, HBTU, Et$_2$^{i}PrN, CHCl$_3$, rt, 24 h 58%; xv. TFA, TIS, reflux, 3.5 h 91%.

Crystallisation of compound **2** from methanol/water afforded one colourless plate (0.32 × 0.27 × 0.08 mm) from which a single crystal X-ray structure was determined. This structure reveals *trans* geometry about both amide bonds, and the *S*-methyl groups oriented away from the pyridine nitrogen (Figure 3 and Supporting Information).

Figure 3. X-Ray crystal structure of pyridine-2,6-dicarboxylic acid bis(*S*-methyl-L-cysteine methyl ester)carboxamide (**2**). Carbon atoms are shown in grey, oxygen in red, nitrogen in lilac, sulfur in yellow and hydrogen in white. CCDC-1017988 contains the full supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk/data_request/cif.

2.2. Nitrile Hydration Experiments

The ability of ligands **1–4** to promote nitrile hydration was assessed under a range of conditions adapted from those reported previously by Chottard and Mascharak to achieve biomimetic nitrile hydration with small-molecule systems [4,35,37,39]. Thus different permutations of ligand **1–4**, metal ion (iron(II), iron(III), cobalt(II) and cobalt(III) salts), base (to effect ligand deprotonation and moderate pH), oxidant (to bring about *S*-oxidation) and nitrile substrate were combined *in situ* (Table 1). Acetonitrile **17** was used throughout screening experiments, present in excess as both substrate and co-solvent. Turnover reaction products were analysed by gas chromatography (GC) in comparison to authentic samples of the starting material **17** and expected product acetamide **18**; turnover was quantified using the single point internal standard method [55,56]. See Supporting Information for details of GC conditions and representative chromatograms (Supplementary Figures S1–S4).

Table 1. Summary of variables screened in nitrile turnover experiments.

Ligand	Metal Salt	Temperature (°C)	pH	Oxidant
1	$Fe(OAc)_2$			
	$Fe(NO_3)_3$	0	2	
2				$+H_2O_2$
	$Na_3[Co(NO_2)_6]$	20	5	
3				$-H_2O_2$
	$CoCl_2 \cdot 6H_2O$	50	9	
4				
	$[Co(NH_3)_5Cl]Cl_2$			

Turnover experiments were typically conducted by dissolving the ligand in acetonitrile, treating with aqueous sodium hydroxide (four equivalents for thiolate ligands **1** and **4** (2 × SH, 2 × NH, anticipating that the acidity of the amide protons would be increased by *N*-coordination to the metal); two equivalents for thioethers **2** and **3**) then adding the metal salt to effect complexation. The resulting solution was adjusted to the required pH using hydrochloric acid or aqueous sodium hydroxide solution, and hydrogen peroxide (3 equivalents) was added to one series of experiments with a view to achieving *S*-oxidation *in situ* [34,57]. The mixture was then adjusted to the required temperature and stirred for the allotted time (12–72 h). All turnover reactions were carried out in triplicate in order to obtain consistent results, thus turnover data discussed below are the average of at least three discrete experiments.

A comprehensive range of control experiments was carried out: (i) no metal salt; (ii) no ligand; (iii) no base; and (iv) no oxidant, to test for direct nitrile hydration by the free ligand (thiols are known to increase the rate of nitrile hydrolysis under certain conditions [58]) the metal (since cobalt(III) can directly hydrolyse nitriles in solution [59]), the added base (to control for direct hydrolysis by hydroxide, and by thiolate or metal-bound hydroxide species) and the oxidant (to account for the possibility of direct reaction between nitrile and peroxide). Figure S2 (Supporting Information) shows the chromatogram arising from a typical control experiment. Turnover was only observed when both metal and ligand were present (Table 2).

Ligands **1** and **2** returned no nitrile hydration under most of the conditions screened, and only very low levels of reaction at their best: 0.20 and 0.40 turnovers respectively (*i.e.*, sub-stoichiometric amide formation) achieved using $Na_3[Co(NO_2)_6]$/pH 9/*no* H_2O_2/50 °C/72 h with **1** and $Fe(NO_3)_3$/pH 9/H_2O_2 (3 eq.)/20 °C/72 h with **2**. The control experiments outlined above confirmed that the specific

combinations of ligand, metal, base and oxidant (in the case of **2**) were required to mediate turnover. The methionine ligand **3** did not turn over acetonitrile under any of the conditions tested.

Table 2. Summary of optimal conditions for acetonitrile turnover with ligands **1–4**.

Ligand	Optimal Turnover Conditions					Turnovers
1	$Na_3[Co(NO_2)]_6$	50 °C	pH 9	72 h	$-H_2O_2$	0.20
2	$Fe(NO_3)_3$	20 °C	pH 9	72 h	$+H_2O_2$	0.40
3	–	–	–	–	–	–
4	$Na_3[Co(NO_2)]_6$	25 °C	pH 9	12 h	$-H_2O_2$	1.25

Ligand **4** returned the best turnover results found with these systems, when combined with $Na_3[Co(NO_2)_6]$ and stirred for 12 h at 25 °C and pH 9 (Scheme 2 shows the reaction outcome, Figure S3 (Supporting Information) shows a representative chromatogram for this level of turnover). The geminal dimethyl unit adjacent to the thiolate enhances the sub-stoichiometric turnover seen with **1** to a gently catalytic level of 1.25 turnovers. The addition of peroxide diminishes catalytic activity with this ligand (down to 0.7 turnovers), while increasing reaction temperature to 50 °C also lowers activity to 0.7 turnovers.

Scheme 2. Optimised conditions for nitrile hydration using ligand **4**.
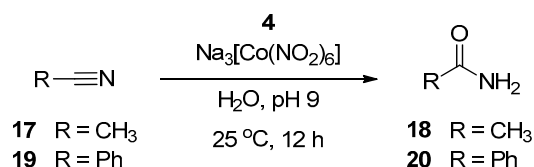
To investigate the scope of this ligand, a further set of experiments was carried out using a second substrate, the aromatic nitrile benzonitrile **19**. Under these conditions the combination of ligand **4** and $Na_3[Co(NO_2)_6]$ also turned over benzonitrile **19** as a substrate, with a similar level of conversion to benzamide **20** (1.2 turnovers). (To aid the miscibility of the benzonitrile in water, a small amount of DMF was added to these reactions: 200 µL in a total reaction volume of 7.2 mL).

3. Experimental Section

3.1. General Information

Chemicals were supplied by Sigma-Aldrich (Castle Hill, NSW, Australia), Merck Millipore (Bayswater, Vic, Australia) or Bio-Scientific (Kirrawee, NSW, Australia). All reactions were carried out under a dry nitrogen or argon atmosphere unless otherwise indicated. Analytical thin-layer chromatography (TLC) was performed on Merck Kieselgel 0.25 mm silica gel 60 F254 plates (Merck Millpore). TLC plates were visualised using UV fluorescence at 254 nm, or by staining with Goofy's reagent (6:6:194:1 phosphomolybdic acid–conc. sulphuric acid–water–cerium sulfate). Flash chromatography on silica gel was performed using a force flow of the indicated solvent system on Ajax Finechem silica gel (230–400 mesh).

Solvents used for extraction and chromatography were distilled prior to use. Where necessary, solvents and reagents were dried over 4 Å molecular sieves prior to use. Tetrahydrofuran was distilled anaerobically from sodium wire. Dichloromethane, methanol, acetonitrile, diisopropylethylamine and triethylamine were distilled from calcium hydride. Dimethylformamide was distilled and stored over 4 Å molecular sieves. Methyl iodide was purified by passage through neutral alumina. Anisole and benzonitrile and were purified by distillation under reduced pressure. Benzamide was recrystallised from water. All other commercial reagents were used as received without further purification.

Melting points were recorded on a Gallenkamp melting point apparatus (Sanyo Gallenkamp, Loughborough, UK). ^{1}H- and ^{13}C-NMR spectra were recorded on Bruker Avance DPX200, DPX300 and DPX400 spectrometers (Bruker, Billerica, MA, USA). Chemical shifts (δ) are reported in ppm from tetramethylsilane (TMS), using TMS or the solvent resonance as the internal reference. Low resolution mass spectra were obtained on a Finnigan LCQ MS Detector (ESI, APCI) (Thermo Fisher Scientific, Waltham, MA, USA). High resolution mass spectra were obtained on a Fisons VG Tofspec (MALDI-TOF) (Thermo Fisher Scientific). Optical rotations were measured on a Bio-Rad FTS-40 polarimeter (Bio-Rad Laboratories Inc., Hercules, CA, USA) with sodium lamp operating at 589 nm using a 1 dm cell and concentrations (c) measured in 100 mg/mL. IR spectra were measured at room temperature on a Bio-Rad Shimadzu 8400S (Bio-Rad Laboratories Inc.) using a sodium chloride cell, NaCl plates or KBr disc. Wavenumbers are given in reciprocal cm and bands are expressed as strong (s), medium (m) and weak (w). Gas chromatography was carried out on a Hewlett Packard 5890A gas chromatograph (Hewlett Packard, Palo Alto, CA, USA). The column used was a SGE 25QC2/BPX with an internal diameter of 0.25 nm.

3.2. Preparation of Protected Amino Acids

General Methyl Esterification Procedure [50]

Amino acids/protected amino acids were treated with thionyl chloride (2 equivalents) in methanol, heated at reflux for 4 h and then stirred for 16 h at room temperature. The solvent was removed *in vacuo* and the crude product recrystallized from diethyl ether to yield the methyl ester as its hydrochloride salt in high yield and high purity.

S-p-Methoxybenzyl-L-cysteine methyl ester (**11**) [49,50]. L-Cysteine (**6**, 5.61 g, 46.3 mmol) was dissolved in TFA (15 mL) and DCM (50 mL), cooled to 0 °C and stirred while a solution of *p*-methoxybenzyl chloride (7.22 g, 46.1 mmol) in DCM (50 mL) was added dropwise. The reaction mixture was stirred at 0 °C for 1 h then allowed to warm to room temperature and stirred for 18 h. Methanol (20 mL) was added and the product was extracted into water (2 × 70 mL). The aqueous phase was washed with DCM (70 mL) and solid impurities removed by filtration. The filtrate was concentrated *in vacuo* to afford *S-p*-methoxybenzyl-L-cysteine trifluoroacetate salt as a white solid (15.24 g, 93%); this intermediate (15.24 g, 42.9 mmol) was dissolved in methanol (100 mL) and thionyl chloride (5.0 mL, 68.5 mmol) added drop-wise. The reaction mixture was heated at reflux for 18 h before the solvent was removed *in vacuo* to yield an off-white solid. This crude product was partitioned between ethyl acetate (40 mL) and saturated aqueous NaHCO$_3$ (20 mL). The organic phase was washed with a further aliquot of saturated aqueous NaHCO$_3$ (20 mL), water (40 mL) and brine (40 mL) then dried (MgSO$_4$). The

solvent was removed *in vacuo* to yield **11** as viscous brown oil (8.42 g, 77%); ν_{max} (thin film): 3364, 2950, 2837, 2363, 1734, 1643, 1551, 1450, 1300; δ_H (CDCl$_3$, 300 MHz) 2.70 (1H, dd, J = 13.5, 7.5), 2.84 (1H, dd, J = 13.5, 4.5), 3.70 (2H, s), 3.72 (3H, s), 3.79 (3H, s), 4.62–4.68 (1H, m), 5.31 (1H, br s), 6.84 (2H, d, J = 8.5), 7.22 (2H d, J = 8.5); δ_C (CDCl$_3$, 75 MHz) 36.0, 36.3, 52.7, 54.0, 55.3, 114.0, 130.0, 150.2, 158.7, 174.4; *m/z* (ESI+) 256 (100%, [M+H]$^+$). Data in agreement with literature [60].

S-Methyl-L-cysteine methyl ester hydrochloride salt (**12**) [48]. L-Cysteine (**6**, 4.20 g, 35.0 mmol) was dissolved in absolute ethanol (90 mL). Sodium metal (2.75 g, 120.0 mmol) was added slowly to the solution at 0 °C. The reaction mixture was allowed to stir for a further 30 min. Methyl iodide (2.10 mL, 26.0 mmol, purified by passing down a short column of alumina prior to use) was added drop-wise to the reaction at 0 °C. The mixture was allowed to warm to room temperature over a period of 30 min. The solution was adjusted to pH 5 using HCl (10 M) before diethyl ether (80 mL) was added, affording a cloudy white suspension. The mixture was filtered under vacuum and the isolated solid was washed with ether (100 mL) and dried under vacuum to give *S*-methyl-L-cysteine (4.00 g, 85%). A portion of this solid (0.56 g, 4.40 mmol) was subjected to the general methyl esterification conditions using thionyl chloride (0.65 mL, 8.80 mmol) in methanol (10 mL) to give the title compound **12** as a white solid (0.82 g, 85%); ν_{max} (KBr pellet): 3362 (s), 1745 (s), 1606 (s); δ_H (300 MHz, D$_2$O): 2.10 (3H, s), 3.01 (1H, dd, J = 15.0, 7.5 Hz), 3.13 (1H, dd, J = 15.0, 4.5 Hz), 3.81 (3H, s), 4.30 (1H, dd, J = 7.5, 4.5 Hz); δ_C (75.4 MHz, CDCl$_3$): 16.2, 34.1, 52.7, 53.6, 168.8; *m/z* (ES$^+$): 150 (15%, [M+H]$^+$). Data in agreement with literature [61].

L-Methionine methyl ester hydrochloride salt (**13**) [50]. Using the general methyl esterification conditions, L-methionine (**8**, 2.00 g, 13.4 mmol) was treated with thionyl chloride (2.00 mL, 26.8 mmol) in methanol (20 mL) to afford the title compound as a white powder (1.95 g, 89%); ν_{max} (KBr pellet): 3080 (s), 1747 (s), 2840 (w), 1236 (s); δ_H (300 MHz, CDCl$_3$): 2.28 (3H, s), 2.28–2.33 (2H, m), 2.67–2.77 (2H, m), 3.87 (3H, s), 4.16–4.30 (1H, m); δ_C (75.4 MHz, CD$_3$OD): 15.4, 30.5, 31.1, 53.2, 54.2, 171.2; *m/z* (ES$^+$): 164 (100%, [M+H]$^+$). Data in agreement with literature [62].

S-p-Methoxybenzyl-L-penicillamine methyl ester (**14**) [51]. To a solution of L-penicillamine (**9**, 0.25 g, 1.60 mmol) in glacial acetic acid (5.0 mL) was added *p*-methoxybenzyl chloride (0.24 mg, 227 µL, 1.55 mmol). The resulting mixture was heated at 80 °C for 72 h then the solvent was removed *in vacuo*. The oily residue was dissolved in acetonitrile (5.0 mL) and the solvent was removed *in vacuo*. This procedure was repeated twice more to reveal a white solid. This solid was dissolved in a solution of diisopropylethylamine in methanol (10% v/v, 12.0 mL) and stirred. Di-*tert*-butyl-dicarbonate (0.71 g, 3.25 mmol) was added in a single portion. The reaction mixture was heated at reflux for 3 h and then left to stir at room temperature overnight. The solvent was removed *in vacuo* and the oily residue was dissolved in ethyl acetate (50 mL). This solution was washed with aqueous HCl (1 M, 40 mL), water (40 mL) and brine (40 mL), then dried over MgSO$_4$, and the solvent was removed *in vacuo* to reveal *N-tert*-butyloxycarbonyl-*S*-*p*-methoxybenzyl-L-penicillamine (**10**) as a white solid (0.53 g, 89%); δ_H (300 MHz, CD$_3$CN): 1.34 (3H, s), 1.41 (3H, s), 1.42 (9H, s), 3.77 (3H, s), 3.73–3.75 (2H, m), 4.20 (1H, br d, J = 8.5 Hz), 5.62 (1H, br d , J = 8.5 Hz), 6.85 (2H, d, J = 8.5 Hz), 7.23 (2H, d, J = 8.5 Hz); δ_C (75.4 MHz, CD$_3$CN): 25.6, 27.1, 28.6, 33.1, 48.1, 56.2, 61.2, 80.2, 114.7, 130.6, 131.2, 159.8, 168.2, 172.2; *m/z* (ES$^-$):

368 ([M–H]⁻, 90%), 737 ([2M–H]⁻, 100%). Compound **10** (1.55 g, 4.21 mmol) and NaHCO₃ (0.71 g, 8.40 mmol) were dissolved in DMF (20 mL) and stirred while methyl iodide (1.20 g, 524 µL, 8.40 mmol) was added. The mixture was stirred overnight. The solvent was removed *in vacuo* and the crude residue redissolved in ethyl acetate (20 mL) and washed with saturated aqueous NaHCO₃ (10 mL), water (10 mL) and brine (10 mL). The organic phase was dried over MgSO₄ and the solvent removed *in vacuo*. The residue was combined with *p*-toluenesulfonic acid (0.54 g, 4.20 mmol) in dichloromethane (25 mL) and heated at reflux for 4 h. The resulting solution was cooled to room temperature then washed with saturated aqueous NaHCO₃ (10 mL), water (10 mL) and brine (10 mL). The organic phase was dried over MgSO₄ and evaporated *in vacuo* to yield the title compound as a clear oil (0.70 g, 59%); $[\alpha]_D^{20}$ = +90.4 (*c* = 0.66 in EtOAc); ν_{max} (thin film) 3378 (s), 1740 (s), 834 (s); δ_H (300 MHz, CDCl₃): 1.29 (3H, s), 1.42 (3H, s), 1.77 (2H, s), 3.49 (1H, s), 3.71 (1H, s), 3.73 (3H, s), 3.76 (1H, s), 3.78 (3H, s), 6.83 (2H, d, *J* = 5.5 Hz), 7.23 (2H, d, *J* = 5.5 Hz); δ_C (75.4 MHz, CDCl₃): 24.0 26.6, 32.8, 50.2, 51.9, 55.4, 61.8, 110.8, 114.2, 130.2, 159.0, 173.9; *m/z* (ES+): 284 ([M+H]⁺, 100%); HRMS (ES⁺): [M+H]⁺ C₁₄H₂₂NO₃S requires 284.1315, found 284.1325.

3.3. Peptide Coupling Method

Amino acid methyl ester (as free amine or HCl salt, 0.2–1.8 g, 2 eq.) pyridine-2,6-dicarboxylic acid (**5**, 1 eq.) and *N,N,N′,N′*-tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate (HBTU, 2 eq.) were dissolved in DCM or chloroform (20–40 mL) and triethylamine or DIPEA (2 eq. for free amine, 4 eq. for HCl salt) was added. The reaction mixture was stirred at room temperature for 22–48 h while monitored by TLC. Additional DCM or chloroform (10–20 mL) was added and the solution washed with equivalent volumes of water, 1 M hydrochloric acid, saturated aqueous sodium hydrogen carbonate, and brine. The organic phase was dried over MgSO₄ then concentrated *in vacuo* to give the crude product (generally a yellow oil) which was purified by column chromatography.

3.4. Ligand Synthesis

Pyridine-2,6-dicarboxylic acid bis(S-p-methoxybenzyl-L-cysteine methyl ester) carboxamide (**15**). Pyridine-2,6-dicarboxylic acid (**5**, 0.06 g, 0.39 mmol) and *S*-*p*-methoxybenzyl-L-cysteine methyl ester (**11**, 0.20 g, 0.78 mmol) were coupled to give **15** as a thick, colourless oil (0.21 g, 42%) after purification by column chromatography (EtOAc–petroleum ether 2:1); R_f 0.30 (EtOAc–petroleum ether 2:1); $[\alpha]_D^{20}$ = −44.8 (*c* = 0.5, CHCl₃); ν_{max} (CHCl₃): 3501 (w), 1742 (s), 1736 (s); δ_H (300 MHz, CDCl₃): 3.01 (4H, d, *J* = 5.5 Hz), 3.62–3.84 (16H, overlapping m and 2 × s), 4.96 (2H, dd, *J* = 18.5, 7.5 Hz), 6.77 (4H, d, *J* = 8.5 Hz), 7.20 (4H, d, *J* = 8.5 Hz), 8.05 (1H, t, *J* = 7.5 Hz), 8.35 (2H, d, *J* = 7.5 Hz), 8.57 (2H, d, *J* = 7.5 Hz); δ_C (75.4 MHz, CDCl₃): 33.2, 36.0, 51.7, 52.7, 55.2, 113.9, 125.3, 129.4, 130.0, 139.1, 148.2, 158.7, 163.1, 171.0; *m/z* HRMS (ES⁺): C₃₁H₃₅N₃O₈S₂Na⁺ ([M+Na]⁺) requires 664.1763, found 664.1761.

Pyridine-2,6-dicarboxylic acid bis(S-methyl-L-cysteine methyl ester)carboxamide (**2**). Pyridine-2,6-dicarboxylic acid (**5**, 0.23 g, 1.4 mmol) and *S*-methyl-L-cysteine methyl ester hydrochloride salt (**12**, 0.50 g, 2.7 mmol) were combined to give **2** as a pale yellow oil (0.39 g, 68%) after purification by column chromatography (EtOAc–cyclohexane 2:1); R_f 0.40 (EtOAc–cyclohexane 2:1); $[\alpha]_D^{20}$ = −8.0 (*c* = 1.0, CHCl₃); ν_{max} (thin film) 3354 (s), 3043 (w), 1743 (s), 1678 (s); δ_H (300 MHz, CDCl₃): 2.19

(6H, s), 2.84 (4H, d, J = 3.5 Hz), 3.64 (6H, s), 5.02 (2H, dt, J = 8.0, 5.5 Hz), 8.07 (1H, t, J = 8.0 Hz), 8.37 (2H, d, J = 8.0 Hz), 8.64 (2H, d J = 8.0 Hz); δ_C (75.4 MHz, CDCl$_3$): 16.1, 36.4, 51.6, 52.8, 125.8, 139.5, 148.7, 163.6, 171.4; *m/z* (ES$^+$): 452 (30%, [M+Na]$^+$), 430 (100%, [M+H]$^+$); HRMS (ES$^+$): C$_{17}$H$_{24}$N$_3$O$_6$S$_2$ ([M+H]$^+$) requires 430.1107, found 430.1118.

Pyridine-2,6-dicarboxylic acid bis(L-methionine methyl ester)carboxamide (**3**). Pyridine-2,6-dicarboxylic acid (**5**, 0.92 g, 5.5 mmol) and L-methionine methyl ester hydrochloride salt (**13**, 1.80 g, 11.0 mmol) were united to give **3** as a yellow oil (0.81 g, 33%) after purification by column chromatography (DCM: EtOAc 4:1); R$_f$ 0.30 (DCM: EtOAc 4:1); $[\alpha]_D^{20}$ = −10.3 (*c* = 1.0, CHCl$_3$); ν_{max} (thin film): 3392 (s), 1739 (s), 1677 (s); δ_H (300 MHz, CDCl$_3$): 2.08 (6H, s), 2.12–2.24 (4H, m), 3.05–3.16 (4H, m), 3.74 (6H, s), 4.87–4.89 (2H, m), 7.92–8.02 (1H, t, J = 7.5 Hz), 8.29 (2H, d, J = 7.5 Hz), 8.57 (2H, d, J = 8.0 Hz); δ_C (75.4 MHz, CDCl$_3$): 15.7, 30.6, 31.5, 52.3, 52.9, 125.9, 139.1, 147.0, 163.7, 172.9; *m/z* (ES$^+$): 458 (100%, [M+H]$^+$); HRMS (ES$^+$): C$_{19}$H$_{28}$N$_3$O$_6$S$_2$ ([M+H]$^+$) requires 458.1417, found 458.1409. Data in agreement with literature [54].

Pyridine-2,6-dicarboxylic acid bis(S-p-methoxybenzyl-L-penicillamine methyl ester) carboxamide (**16**). Pyridine-2,6-dicarboxylic acid (**5**, 0.16 g, 0.97 mmol) and *S-p*-methoxybenzyl-L-penicillamine methyl ester (**14**, 0.55 g, 1.94 mmol) were joined to give **16** as a clear yellow oil (0.39 g, 58%) after purification by column chromatography (hexane–EtOAc 6:4); R$_f$ 0.25 (hexane–EtOAc 6:4); $[\alpha]_D^{20}$ = +2.1 (*c* = 1.12, EtOAc); ν_{max} (KBr): 3401 (m), 1742 (m), 1681 (m); δ_H (300 MHz, CDCl$_3$): 1.26 (6H, s), 1.48 (6H, s), 3.62 (6H, s), 3.73 (2H, s), 3.74 (1H, s), 3.76 (6H, s), 4.75 (2H, d, J = 7.5 Hz), 6.71 (4H, d, J = 8.5 Hz), 7.18 (4H, d, J = 8.5 Hz), 8.05 (1H, t, J = 7.5 Hz), 8.35 (2H, d, J = 8.0 Hz), 8.62 (2H, d, J = 7.5 Hz); δ_C (75.4 MHz, CDCl$_3$): 25.0, 27.7, 33.4, 48.8, 52.8, 55.6, 60.5, 114.6, 126.3, 129.3, 131.1, 139.2, 148.3, 160.0, 164.8, 171.5; *m/z* (ES+): 720 ([M+Na]$^+$, 100%); HRMS (ES+): C$_{35}$H$_{44}$N$_3$O$_8$S$_2$ ([M+H]$^+$) requires 698.2587, found 698.2595.

Pyridine-2,6-dicarboxylic acid bis(L-cysteine methyl ester)carboxamide (**1**). Pyridine-2,6-dicarboxylic acid bis(*S-p*-methoxybenzyl-L-cysteine methyl ester)carboxamide (**15**, 100 mg, 0.15 mmol) and triisopropylsilane (TIS, 195 μL, 0.95 mmol) were dissolved in TFA (1.25 mL). The reaction mixture was heated at reflux for 2.5 h after which time the solvent was removed *in vacuo*. The residue was washed with ether (3 × 2 mL). The crude product was purified by flash column chromatography (EtOAc–petroleum ether 2:1) to afford the pure product **1** as white thick oil (41 mg, 63%) which was stored and handled under nitrogen to minimize disulfide formation; R$_f$ 0.20 (ether: hexane 1:1); $[\alpha]_D^{20}$ = +8.0 (*c* = 0.1, CHCl$_3$); ν_{max} (thin film): 3394 (s), 2570 (w), 1739 (s), 1670 (s); δ_H (300 MHz, CDCl$_3$): 1.55 (2H, t, J = 9.0 Hz), 3.14–3.20 (4H, m), 3.84 (6H, s), 5.07–5.09 (2H, m), 8.08 (1H, t, J = 7.5 Hz), 8.37 (2H, d, J = 7.5 Hz), 8.66 (2H, d, J = 7.0 Hz); δ_C (75.4 MHz, CDCl$_3$): 27.0, 52.9, 53.6, 125.5, 139.3, 148.2, 163.1, 170.1; *m/z* (ES$^+$): 402 (100%, [M+H]$^+$), 424 (25%, [M+Na]$^+$); HRMS (ES$^+$): C$_{15}$H$_{20}$N$_3$O$_6$S$_2$ ([M+H]$^+$) requires 402.0791, found 402.0795.

Pyridine-2,6-dicarboxylic acid bis-(L-penicillamine methyl ester) carboxamide (**4**). Pyridine-2,6-dicarboxylic acid bis-*S-p*-methoxybenzyl-L-penicillamine methyl ester (**16**, 0.40 g, 0.57 mmol) and TIS (720 μL, 3.5 mmol) were dissolved in TFA (5.0 mL). The reaction mixture was heated at reflux for 2.5 h. The solvent was removed *in vacuo* and the residue triturated with ether and hexane. Flash column

chromatography of the crude material (hexane–EtOAc 3:2) furnished **4** as a clear yellow oil (0.24 g, 91%); R_f 0.30 (hexane–EtOAc 3:2); $[\alpha]_D^{20}$ = +2.1 (*c* = 0.04, EtOAc); ν_{max} (CHCl$_3$): 1743 (s), 1667 (s); δ_H (300 MHz, CDCl$_3$): 1.52 (6H, s), 1.62 (6H, s), 2.22 (2H, br s), 3.82 (6H, s), 4.83 (2H, d, *J* = 9.5 Hz), 8.06 (1H, t, *J* = 8.0 Hz), 8.34 (2H, d, *J* = 8.0 Hz), 8.75 (2H, d, *J* = 9.5 Hz); δ_C (75 MHz, CDCl$_3$): 29.8, 30.8, 46.7, 52.3, 61.0, 125.6, 139.31, 148.2, 163.2, 170.4; *m/z* (ES+): 480 (10%, [M+Na]$^+$); HRMS (ES$^+$): C$_{19}$H$_{27}$N$_3$NaO$_6$S$_2$ ([M+Na]$^+$) requires 480.1237, found 480.1238.

3.5. Crystallography

A colourless plate-like crystal of **2** was attached with Exxon Paratone N, to a short length of fibre supported on a thin piece of copper wire inserted in a copper mounting pin. The crystal was quenched in a cold nitrogen gas stream from an Oxford Cryosystems Cryostream (Oxford Cryosystems Ltd, Long Hanborough, UK). A Bruker-Nonius FR591 Kappa APEX II diffractometer (Bruker Analytical X-ray Instruments Inc., Madison, WI, USA) employing graphite monochromated MoKα radiation generated from a fine-focus rotating anode was used for the data collection. Cell constants were obtained from a least squares refinement against 7789 reflections located between 5.68 and 65.16° 2θ. Data were collected at 150(2) Kelvin with ϕ and ω scans to 65.18° 2θ. The data integration and reduction were undertaken with SAINT (Bruker Analytical X-ray Instruments Inc.) and XPREP (Bruker Analytical X-ray Instruments Inc.) [63], and subsequent computations were carried out with the X-Seed graphical user interface (University of Missouri, Columbia, MO, USA) [64]. An empirical absorption correction determined with SADABS (University of Göttingen, Göttingen, Germany) was applied to the data [65,66].

The structure was solved in the space group P1(#1) by direct methods with SHELXS-97 (University of Göttingen), and extended and refined with SHELXL-97 [67]. The non-hydrogen atoms in the asymmetric unit were modelled with anisotropic displacement parameters. A riding atom model with group displacement parameters was used for the hydrogen atoms. The absolute structure was established with the Flack parameter [68–71] refining to −0.01(3).

3.6. Turnover Experiments

3.6.1. General Procedure

The ligand (0.10 mmol) was dissolved in the nitrile (5.0 mL) and sodium hydroxide (2–4 eq., 0.20–0.40 mmol) was added. The metal salt (0.10 mmol) was added and the mixture stirred at ambient temperature for 15 min. If oxidant was to be included, the solution was cooled to 0 °C while H$_2$O$_2$ (30%, 0.30 mmol) was added dropwise with stirring, and the mixture was stirred on ice for 30 min. Water (2.0 mL) was added and the mixture adjusted to the required pH (2, 5 or 9, using aqueous HCl or NaOH) and temperature (0, 20 or 50 °C) then stirred for 12–72 h. The solvent was removed *in vacuo*, the residue re-dissolved in acetonitrile (1 mL) and the solution filtered through a plug of silica to remove particulates. The sample was analysed by gas chromatography against an internal standard, and product(s) were identified by comparison to authentic samples.

3.6.2. GC Analysis

Calibration of the internal standard/ relative response factor was carried out using the single point internal standard method [55,56]. Thus equimolar quantities of anisole and acetamide in ethyl acetate (1 μL) were injected into the GC and the peak area for each was recorded. Three injections were made and an average of the three measurements taken. Under the conditions used (Table S1, Supporting Information) residual acetonitrile **17** eluted with the solvent front. Anisole, the internal standard, eluted with a retention time (R_t) of 6.88 ± 0.03 min, while the product acetamide **18** eluted at R_t 12.64 ± 0.06 min (Figure S1). The relative response factor (the relative peak area of the two components) was 3.35 (*i.e.*, the area of the internal standard was 3.35 times greater than the area of the acetamide product when present in equal molar quantities). By adding a defined quantity of anisole to turnover reaction mixtures immediately prior to analysis by GC and using the relative response factor (3.35), the amount of acetamide formed could be accurately quantified, even at <0.1 turnovers. Further details of GC run times and temperatures, calculation of internal response factors and example chromatograms are provided in the Supporting Information (Tables S1 and S2, Figures S1–S4).

4. Conclusions

We have prepared a series of pentadentate ligands that combine sulfur-containing amino acids on a pyridine-2,6-dicarboxylic acid scaffold to render an N_3S_2 donor set incorporating key elements of the NHase active site. Evaluating the iron(III)- and cobalt(III)-complexes of these ligands as catalysts for nitrile hydration demonstrates low levels of turnover (1.20–1.25 turnovers) for the penicillamine ligand **4** in combination with cobalt(III), with both acetonitrile and benzonitrile substrates. Placed in context, the most active small-molecule mimics of NHase catalysis to date employ preformed cobalt complexes under optimised conditions to achieve 15–18 turnovers for a non-oxidised pyridine dithiolate ligand (at 50 °C and pH 9.5 in Tris buffer) [35], and 50 turnovers with a bis-sulfenate ligand (at 4 °C and pH 4.8 in acetate buffer) [37]. Future work will investigate the importance of the pyridine nitrogen to the catalytic activity of ligand **4**.

Supplementary Materials

Supplementary materials can be accessed at: http://www.mdpi.com/1420-3049/19/12/20751/s1.

Author Contributions

C.B., K.M.H. and P.J.R. conceived and designed the experiments; C.B., K.M.H., P.D. and P.J. (crystallography) performed the experiments; P.J. (crystallography), C.B., K.M.H., P.D. and P.J.R. analyzed the data; C.B., K.M.H. and P.J.R. wrote the paper.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Garcia-Alvarez, R.; Crochet, P.; Cadierno, V. Metal-catalyzed amide bond forming reactions in an environmentally friendly aqueous medium: Nitrile hydrations and beyond. *Green Chem.* **2013**, *15*, 46–66.
2. Ahmed, T.J.; Knapp, S.M.M.; Tyler, D.R. Frontiers in catalytic nitrile hydration: Nitrile and cyanohydrin hydration catalyzed by homogeneous organometallic complexes. *Coord. Chem. Rev.* **2011**, *255*, 949–974.
3. Kukushkin, V.Y.; Pombeiro, A.J.L. Metal-mediated and metal-catalyzed hydrolysis of nitriles. *Inorg. Chim. Acta* **2005**, *358*, 1–21.
4. Kumar, D.; Masitas, C.A.; Nguyen, T.N.; Grapperhaus, C.A. Bioinspired catalytic nitrile hydration by dithiolato, sulfinato/thiolato, and sulfenato/sulfinato ruthenium complexes. *Chem. Commun.* **2013**, *49*, 294–296.
5. Cadierno, V.; Diez, J.; Francos, J.; Gimeno, J. Bis(allyl)ruthenium(IV) complexes containing water-soluble phosphane ligands: Synthesis, structure, and application as catalysts in the selective hydration of organonitriles into amides. *Chem. Eur. J.* **2010**, *16*, 9808–9817.
6. Polshettiwar, V.; Varma, R.S. Nanoparticle-supported and magnetically recoverable ruthenium hydroxide catalyst: Efficient hydration of nitriles to amides in aqueous medium. *Chem. Eur. J.* **2009**, *15*, 1582–1586.
7. Cadierno, V.; Francos, J.; Gimeno, J. Selective ruthenium-catalyzed hydration of nitriles to amides in pure aqueous medium under neutral conditions. *Chem. Eur. J.* **2008**, *14*, 6601–6605.
8. Goto, A.; Endo, K.; Saito, S. Rh(I)-catalyzed hydration of organonitriles under ambient conditions. *Angew. Chem. Int. Edit.* **2008**, *47*, 3607–3609.
9. Djoman, M.; Ajjou, A.N. The hydration of nitriles catalyzed by water-soluble rhodium complexes. *Tetrahedron Lett.* **2000**, *41*, 4845–4849.
10. Hirano, T.; Uehara, K.; Kamata, K.; Mizuno, N. Palladium(II) containing gamma-keggin silicodecatungstate that efficiently catalyzes hydration of nitriles. *J. Am. Chem. Soc.* **2012**, *134*, 6425–6433.
11. Kim, E.S.; Kim, H.S.; Kim, J.N. An efficient Pd-catalyzed hydration of nitrile with acetaldoxime. *Tetrahedron Lett.* **2009**, *50*, 2973–2975.
12. Ghaffar, T.; Parkins, A.W. The catalytic hydration of nitriles to amides using a homogeneous platinum phosphinito catalyst. *J. Mol. Catal. A Chem.* **2000**, *160*, 249–261.

13. Ghaffar, T.; Parkins, A.W. A new homogeneous platinum-containing catalyst for the hydrolysis of nitriles. *Tetrahedron Lett.* **1995**, *36*, 8657–8660.

14. Li, Z.K.; Wang, L.X.; Zhou, X.G. An efficient and practical protocol for catalytic hydrolysis of nitriles by a copper(I) complex in water. *Adv. Synth. Catal.* **2012**, *354*, 584–588.

15. Mitsudome, T.; Mikami, Y.; Mori, H.; Arita, S.; Mizugaki, T.; Jitsukawa, K.; Kaneda, K. Supported silver nanoparticle catalyst for selective hydration of nitriles to amides in water. *Chem. Commun.* **2009**, 3258–3260.

16. Liu, Y.M.; He, L.; Wang, M.M.; Cao, Y.; He, H.Y.; Fan, K.N. A general and efficient heterogeneous gold-catalyzed hydration of nitriles in neat water under mild atmospheric conditions. *ChemSusChem* **2012**, *5*, 1392–1396.

17. Ramon, R.S.; Marion, N.; Nolan, S.P. Gold activation of nitriles: Catalytic hydration to amides. *Chem. Eur. J.* **2009**, *15*, 8695–8697.

18. Tu, T.; Wang, Z.X.; Liu, Z.L.; Feng, X.K.; Wang, Q.Y. Efficient and practical transition metal-free catalytic hydration of organonitriles to amides. *Green Chem.* **2012**, *14*, 921–924.

19. Verma, P.K.; Sharma, U.; Bala, M.; Kumar, N.; Singh, B. Transition metal-free 1,3-dimethylimidazolium hydrogen carbonate catalyzed hydration of organonitriles to amides. *RSC Adv.* **2013**, *3*, 895–899.

20. Lee, J.; Kim, M.; Chang, S.B.; Lee, H.Y. Anhydrous hydration of nitriles to amides using aldoximes as the water source. *Org. Lett.* **2009**, *11*, 5598–5601.

21. Kim, E.S.; Lee, H.S.; Kim, S.H.; Kim, J.N. An efficient InCl₃-catalyzed hydration of nitriles to amides: Acetaldoxime as an effective water surrogate. *Tetrahedron Lett.* **2010**, *51*, 1589–1591.

22. Miyanaga, A.; Fushinobu, S.; Ito, K.; Wakagi, T. Crystal structure of cobalt-containing nitrile hydratase. *Biochem. Biophys. Res. Commun.* **2001**, *288*, 1169–1174.

23. Kobayashi, M.; Shimizu, S. Metalloenzyme nitrile hydratase: Structure, regulation, and application to biotechnology. *Nat. Biotechnol.* **1998**, *16*, 733–736.

24. Kobayashi, M.; Nagasawa, T.; Yamada, H. Enzymatic synthesis of acrylamide: A success story not yet over. *Trends Biotechnol.* **1992**, *10*, 402–408.

25. Padmakumar, R.; Oriel, P. Bioconversion of acrylonitrile to acrylamide using a thermostable nitrile hydratase. *Appl. Biochem. Biotechnol.* **1999**, *77–79*, 671–679.

26. Wang, M.-X. Enantioselective biotransformations of nitriles in organic synthesis. *Top. Catal.* **2005**, *35*, 117–130.

27. Prasad, S.; Bhalla, T.C. Nitrile hydratases (NHases): At the interface of academia and industry. *Biotechnol. Adv.* **2010**, *28*, 725–741.

28. Shigehiro, S.; Nakasako, M.; Dohmae, N.; Tsujimura, M.; Tokoi, K.; Odaka, M.; Yohda, M.; Kamiya, N.; Endo, I. Novel non-heme iron center of nitrile hydratase with a claw setting of oxygen atoms. *Nat. Struct. Biol.* **1998**, *5*, 347–351.

29. Mascharak, P.K. Structural and functional models of nitrile hydratase. *Coord. Chem. Rev.* **2002**, *225*, 201–214.

30. Kovacs, J.A. Synthetic analogues of cysteinate-ligated non-heme iron and non-corrinoid cobalt enzymes. *Chem. Rev.* **2004**, *104*, 825–848.

31. Martinez, S.; Wu, R.; Sanishvili, R.; Liu, D.; Holz, R. The active site sulfenic acid ligand in nitrile hydratases can function as a nucleophile. *J. Am. Chem. Soc.* **2014**, *136*, 1186–1189.

32. Hopmann, K.H.; Himo, F. Theoretical investigation of the second-shell mechanism of nitrile hydratase. *Eur. J. Inorg. Chem.* **2008**, *2008*, 1406–1412.

33. Hopmann, K.H. Full reaction mechanism of nitrile hydratase: A cyclic intermediate and an unexpected disulfide switch. *Inorg. Chem.* **2014**, *53*, 2760–2762.

34. Tyler, L.A.; Noveron, J.C.; Olmstead, M.M.; Mascharak, P.K. Modulation of the pKa of metal-bound water via oxidation of thiolato sulfur in model complexes of Co(III) containing nitrile hydratase: Insight into possible effect of cysteine oxidation in Co-nitrile hydratase. *Inorg. Chem.* **2003**, *42*, 5751–5761.

35. Noveron, J.C.; Olmstead, M.M.; Mascharak, P.K. Co(III) complexes with carboxamido *N* and thiolato *S* donor centers: Models for the active site of Co-containing nitrile hydratases. *J. Am. Chem. Soc.* **1999**, *121*, 3553–3554.

36. Heinrich, L.; Li, Y.; Vaissermann, J.; Chottard, G.; Chottard, J.C. A pentacoordinated di-*N*-carboxamido-dithiolato-*O*-sulfinato-iron(III) complex related to the metal site of nitrile hydratase. *Angew. Chem. Int. Ed.* **1999**, *38*, 3526–3528.

37. Heinrich, L.; Mary-Verla, A.; Li, Y.; Vaissermann, J.; Chottard, J.C. Cobalt(III) complexes with carboxamido-*N* and sulfenato-*S* or sulfinato-*S* ligands suggest that a coordinated sulfenate-*S* is essential for the catalytic activity of nitrile hydratases. *Eur. J. Inorg. Chem.* **2001**, *2001*, 2203–2206.

38. Artaud, I.; Chatel, S.; Chauvin, A.S.; Bonnet, D.; Kopf, M.A.; Leduc, P. Nitrile hydratase and related non-heme iron sulfur complexes. *Coord. Chem. Rev.* **1999**, *192*, 577–586.

39. Bourles, E.; Alves de Sousa, R.; Galardon, E.; Giorgi, M.; Artaud, I. Direct synthesis of a thiolato-*S* and sulfinato-*S* Co(III) complex related to the active site of nitrile hydratase: A pathway to the post-translational oxidation of the protein. *Angew. Chem. Int. Ed.* **2005**, *44*, 6162–6165.

40. Lugo-Mas, P.; Dey, A.; Xu, L.; Davin, S.D.; Benedict, J.; Kaminsky, W.; Hodgson, K.O.; Hedman, B.; Solomon, E.I.; Kovacs, J.A. How does single oxygen atom addition affect the properties of an Fe-nitrile hydratase analogue? The compensatory role of the unmodified thiolate. *J. Am. Chem. Soc.* **2006**, *128*, 11211–11221.

41. Shearer, J.; Jackson, H.L.; Schweitzer, D.; Rittenberg, D.K.; Leavy, T.M.; Kaminsky, W.; Scarrow, R.C.; Kovacs, J.A. The first example of a nitrile hydratase model complex that reversibly binds nitriles. *J. Am. Chem. Soc.* **2002**, *124*, 11417–11428.

42. Krall, J.A.; Rutledge, P.J.; Baldwin, J.E. Design and synthesis of an isopenicillin N synthase mimic. *Tetrahedron* **2005**, *61*, 137–143.

43. Barry, S.M.; Rutledge, P.J. Cis-dihydroxylation of alkenes by a non-heme iron enzyme mimic. *Synlett* **2008**, 2172–2174.

44. Dungan, V.J.; Ortin, Y.; Mueller-Bunz, H.; Rutledge, P.J. Design and synthesis of a tetradentate "3-amine-1-carboxylate" ligand to mimic the metal binding environment at the non-heme iron(II) oxidase active site. *Org. Biomol. Chem.* **2010**, *8*, 1666–1673.

45. Barry, S.M.; Mueller-Bunz, H.; Rutledge, P.J. Investigating the *cis*-dihydroxylation of alkenes by non-heme iron enzyme mimics. *Org. Biomol. Chem.* **2012**, *10*, 7372–7381.

46. Dungan, V.J.; Wong, S.M.; Barry, S.M.; Rutledge, P.J. L-Proline-derived ligands to mimic the "2-his-1-carboxylate" triad of the non-heme iron oxidase active site. *Tetrahedron* **2012**, *68*, 3231–3236.

47. Dungan, V.J.; Poon, B.M.-L.; Barrett, E.; Rutledge, P.J. L-Proline derived mimics of the non-heme iron active site catalyse allylic oxidation in acetonitrile solutions. *Tetrahedron Lett.* **2013**, *54*, 1236–1238.

48. Hwang, D.R.; Helquist, P.; Shekhani, M.S. Total synthesis of (+)-sparsomycin—Approaches using cysteine and serine inversion. *J. Org. Chem.* **1985**, *50*, 1264–1271.

49. Bodanzky, M.; Bodanzky, A. The practice of peptide synthesis. In *Reactivity and Structure Concepts in Organic Chemistry*; Hafner, K., Rees, C.W., Trost, B.M., Lehn, J.M., Schleyer, P.V.R., Zahruchik, R., Eds.; Springer-Verlag: Berlin, Germany, 1984; Volume 21.

50. McMullen, T.C. The methyl ester of orthobenzoyl-benzoic acid. *J. Am. Chem. Soc.* **1916**, *38*, 1228–1230.

51. Bocchi, V.; Casnati, G.; Dossena, A.; Marchelli, R. Esterification of amino acids and dipeptides under mild conditions; part ii: Via sodium salts. *Synthesis* **1979**, 961–962.

52. Han, S.-Y.; Kim, Y.-A. Recent development of peptide coupling reagents in organic synthesis. *Tetrahedron* **2004**, *60*, 2447–2467.

53. Derdau, V.; Laschat, S.; Hupe, E.; König, W.A.; Dix, I.; Jones, P.G. Novel chiral pyridine *N*-oxide ligands and their application in the enantioselective catalytic reduction of ketones and the addition of diethylzinc to aldehydes. *Eur. J. Inorg. Chem.* **1999**, *1999*, 1001–1007.

54. Alexie, M.; Dimitru, F.; Razvan, A.; Pasare, L.; Guran, C. New membranes with mercapto-2,6-pyridinediamides as anion recognition fixed sites. *Rev. Chim.* **2010**, *61*, 1197–1201.

55. Loconto, P.R. *Trace Environmental Quantitative Analysis—Principles, Techniques and Applications*, 2nd ed.; Marcel Dekker Inc.: New York, NY, USA, 2005.

56. Harris, D.C. *Quantitative Chemical Analysis*, 6th ed.; W.H. Freeman: New York, NY, USA, 2003.

57. Heinrich, L.; Li, Y.; Vaissermann, J.; Chottard, J.C. A bis(carboxamido-*N*)diisocyanidobis(sulfenato-*S*)cobalt(III) complex, model for the post-translational oxygenation of nitrile hydratase thiolato ligands. *Eur. J. Inorg. Chem.* **2001**, *2001*, 1407–1409.

58. Cordes, E.H.; Zervos, C. Mercaptoethanol catalysis for hydrolysis of *N*-benzyl-3-cyanopyridinium bromide. A model for the nitrilase reaction. *J. Org. Chem.* **1971**, *36*, 1661–1667.

59. Balahura, R.; Cock, P.; Purcell, W. Kinetics studies of the hydrolysis of coordinated nitriles. *J. Am. Chem. Soc.* **1974**, *96*, 2739–2742.

60. Platen, M.; Steckhan, E. Oxidative deblocking of the 4-methoxybenzyl thioether protecting group: Application to the directed synthesis of poly-cystinyl peptides. *Liebigs Ann. Chem.* **1984**, *1984*, 1563–1576.

61. Matteucci, M.; Bhalay, G.; Bradley, M. Mild and highly chemoselective oxidation of thioethers mediated by Sc(OTf)$_3$. *Org. Lett.* **2003**, *5*, 235–237.

62. Lumbroso, A.; Coeffard, V.; le Grognec, E.; Beaudet, I.; Quintard, J.-P. An efficient and scalable synthesis of *N*-(benzyloxycarbonyl)- and *N*-(methyloxycarbonyl)-(*S*)-vinylglycinol. *Tetrahedron Lett.* **2010**, *51*, 3226–3228.

63. Bruker. *Area Detector Control and Data Integration and Reduction Software*; Bruker Analytical X-ray Instruments Inc.: Madison, WI, USA, 1995.

64. Barbour, L.J. X-seed—A software tool for supramolecular crystallography. *J. Supramol. Chem.* **2001**, *1*, 189–191.

65. Sheldrick, G.M. *Sadabs. Empirical Absorption Correction Program for Area Detector Data*; University of Göttingen: Göttingen, Germany, 1996.

66. Blessing, R.H. An empirical correction for absorption anisotropy. *Acta Crystallogr. Sect. A Found. Crystallogr.* **1995**, *51*, 33–38.

67. Sheldrick, G.M. *Shelx97 Programs for Crystal Structure Analysis*; University of Göttingen: Göttingen, Germany, 1998.

68. Flack, H.D. On enantiomorph-polarity estimation. *Acta Crystallogr. Sect. A Found. Crystallogr.* **1983**, *39*, 876–881.

69. Bernardinelli, G.; Flack, H.D. Least-squares absolute-structure refinement—Practical experience and ancillary calculations. *Acta Crystallogr. Sect. A Found. Crystallogr.* **1985**, *41*, 500–511.

70. Flack, H.D.; Bernardinelli, G. Absolute structure and absolute configuration. *Acta Crystallogr. Sect. A Found. Crystallogr.* **1999**, *55*, 908–915.

71. Flack, H.D.; Bernardinelli, G. Reporting and evaluating absolute-structure and absolute-configuration determinations. *J. Appl. Crystallogr.* **2000**, *33*, 1143–1148.

Sample Availability: Not available.